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(57) Abstract

The present invention teaches the use of antibodies raised against the Group A mucopeptide for the treatment of septic shock and Rheumatoid Arthritis, and the identification of specific peptides that can be used in such treatments. The invention includes methods of using the antibodies raised against the Group A mucopeptide including as a means identifying specific peptides, the specificpeptides themselves, and methods of using the peptides. In certain embodiments, the specific peptides serve as mimics to these antibodies. Such peptides can be administered to an animal subject to block the microbial action that initiates Rheumatoid Arthritis via tumor necrosis factor. Alternatively, specific peptides of the present invention can be used to immunize Rheumatoid Arthritis–susceptible individuals to prevent further progression of the diseases. In still other embodiments, the peptides serve as binding partners for these antibodies.

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METHODS FOR IDENTIFYING ANTIBODIES AND PEPTIDES USEFUL IN THE TREATMENT OF SEPTIC SHOCK AND EXPERIMENTAL ARTHRITIS AND USES THEREOF

FIELD OF THE INVENTION

The present invention relates to methods for identifying new peptides useful in the treatment of septic shock, experimental arthritis and related maladies. These new peptides are selected, in part, for their affinity to or mimicry of anti-Group A mucopeptide antibodies. The therapeutic use of the new peptides and the related antibodies in the treatment of septic shock are disclosed.

BACKGROUND OF THE INVENTION

Studies extending over the years have elucidated the basic structure of many of the gram positive and gram negative organisms. With respect to the gram positive bacteria, the backbone of theses organisms is the polysaccharide-peptide complex called the mucopeptide complex (Figure 1). The basic repeating unit is an alternating motif of Neuraminic Acid-N-acetyl glucosamine (NAM-NAG) molecules in which the inter-peptide bridges vary from organism to organism.

The gram negative bacteria share a common glycolipid commonly known as lipid A, to which various oligo- and polysaccharides are added depending on the bacteria. The common structure of lipid A is shown in Figure 2. It is well known that both Lipid A and the mucopeptide complex will individually elicit an extremely vigorous toxic response when injected into animals. The most serious of these responses is the oftentimes massive production of tumor necrosis factor (TNFα).

Presently, the main approach to blocking this TNF induction has been either the administration of antibodies directed to the TNF molecule itself or to use soluble TNF receptor molecules to competitively inhibit TNF binding to the target cell. Both of these strategies would interrupt cytokine release. Monoclonal antibodies with their restricted epitope specificity have been shown to be unsuccessful in this regard [Fisher *et al.*, *Crit. Care Med.* 21:318-3272 (1994), McCloskey *et al.*, *Ann. Intern. Med.* 121:1-5]. Preliminary trials with $F(ab)_2$ anti-TNF polyclonal antibodies appear to be more promising,

but further trials need to be carried out before such methods are shown to be a viable approach.

The mechanisms for lipid A and the mucopeptide complex triggering of the extremely toxic response, described above, have not been elucidated, but most investigators agree that cell surface receptors on monocytes must be involved. The quest to identify this receptor or receptors is a very active field of research. Over the last several years, Morrison and co-workers, among others, have identified a 70 kDa protein on monocytes which appears to bind to lipopolysaccharides (LPS), [Dziarski, *J.Biol.Chem.* **266**:4719-4725 (1994), Lei *et al.*, *Int.Rev.Immunol.* **6**:223-235 (1990) Lei *et al.*, *J.Immunol.* **147**:1925-1932 (1991) Rabin *et al.*, *J.Infect.Dis.* **168**:135-142, (1993)]. A monoclonal antibody reactive with this 70 kDa protein has been reported to be able to cause monocytes to produce TNF α, which strengthens the proposal that this 70 Kda protein might be the LPS receptor [Morrison *et al.*, *J.Infect.Dis.* **162**:1063-1068 (1990)]. Beyond this putative identification of a receptor, little else has been uncovered concerning this immunologically important mechanism.

Therefore there is a need for a better understanding of the mechanisms of how lipid A and the mucopeptide complex induce their toxic effects. On a more practical side, there is a great need for alternative approaches for blocking the induction of TNFα during septic shock. Thus, there is a also a great need for the design of new pharmaceuticals that can counter-act the toxic effects of lipid A and the mucopeptide complex.

The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

SUMMARY OF THE INVENTION

In its broadest embodiment the present invention teaches the use of antibodies raised against the Group A mucopeptide for the treatment of inflammatory diseases such as septic shock and rheumatoid arthritis, Crohn's disease, psoriasis, and for the identification of specific peptides that can be used in such treatments. In certain embodiments, the specific peptides will serve as mimics to these antibodies. In other embodiments, the peptides will serve as binding partners for these antibodies.

One aspect of the present invention is derived from the premise that there exists a common structural motif for gram positive and gram negative bacteria. The present invention exploits the ramifications of this premise and thereby introduces novel approaches of identifying treatments for septic shock and related diseases.

One feature of the present invention is the generation and identification of antibodies that bind to a common motif present on both Lipid A and the Group A mucopeptide. A related aspect of the present invention is the generation and characterization of peptides that mimic these antibodies. The peptides act by associating with LPS and the Group A mucopeptide, and thus inhibit these bacterial agents from binding to their receptor(s). This, in turn, can prevent the induction of TNFα and the onset of septic shock.

Accordingly, the present invention includes methods of obtaining such inhibitory peptides. In one specific embodiment the inhibitory peptides are obtained by contacting a phage library with permissive bacteria. The phage library contains an assortment of phage with each individual phage comprising a random DNA sequence that encodes specific peptides. The DNA sequences of the phage library are expressed and a variety of random peptides are generated. The random peptides are contacted with the mucopeptide complex, and candidate peptides are selected on the basis of their ability to bind the mucopeptide complex. Phage containing the candidate peptides are purified and DNA that encode the candidate peptides are sequenced. The amino acid sequence for the candidate peptide can then be deduced.

The candidate peptides are synthesized per their respective amino acid sequences. The synthesis can be performed, for example, by standard genetic engineering techniques or more preferably by solid phase peptide synthesis. The synthetic candidate peptides are then contacted with Lipid A, and further characterized by their ability to bind Lipid A. In this manner peptides that bind both Lipid A and the Group A mucopeptide can be obtained. The selected peptides are further contacted with the Group A mucopeptide and an anti-Group A mucopeptide antibody. Inhibitory peptides are chosen from selected peptides on the basis of their distinctive ability to inhibit the formation of that antigen-antibody complex.

It should be clear that though the specific embodiment described above used a phage library to obtain an assortment of random peptides, this assortment also may be generated by chemical synthesis. In addition, the assortment of random peptides can be generated by a combination of these two techniques. It should also be clear that for all of the embodiments provided by the present invention that use an assortment of random peptides, that either a phage library or chemical synthesis can be utilized.

In another embodiment of the present invention, an inhibitory peptide is further selected on the basis of its ability to inhibit the bacterial-mediated production of tumor necrosis factor α (TNFα). This method includes the additional step of contacting an inhibitory peptide with a mononuclear cell that is challenged with either LPS or the mucopeptide. In a preferred embodiment of the method of identifying the inhibitory peptide, the inhibitory peptide binds to a common structural element present in both the Group A mucopeptide and the LPS molecule.

The inhibitory peptides obtained by the methods described herein are also part of the present invention. These inhibitory peptides have the following characteristics: they bind to both the Group A mucopeptide and the LPS molecule; and in addition, inhibit the association between an anti-Group A mucopeptide antibody and its Group A mucopeptide antigen.

In a preferred embodiment, the inhibitory peptide further inhibits the bacterial-mediated production of tumor necrosis factor in a target cell. The target cell can be a mononuclear cell or any other cell which secretes cytokines in response to stimulation by the Group A mucopeptide and/or the LPS molecule. In a more preferred embodiment the mononuclear cell is human.

The present invention also includes pharmaceutical compositions for treating septic shock, rheumatoid arthritis, Crohn's disease, psoriasis and experimental arthritis comprising an inhibitory peptide of the present invention and a pharmaceutically acceptable carrier.

Methods of treating septic shock in mammals, which comprise administering to the mammal a therapeutically effective amount of such pharmaceutical compositions, are included in the

present invention. In preferred embodiments for treating septic shock or rheumatoid arthritis, the mammal is a human.

The present invention also includes methods of treating experimental arthritis comprised of administering to the subject animal a therapeutically effective amount of a pharmaceutical composition comprising an inhibitory peptide of the present invention and a pharmaceutically acceptable carrier. Analogous methods of treating rheumatoid arthritis by administering to a human a therapeutically effective amount of the pharmaceutical compositions comprising an inhibitory peptide of the present invention and a pharmaceutically acceptable carrier are contemplated by the present invention.

A related aspect of the invention is a method of blocking the LPS-mediated induction of TNFα in a mononuclear cell, by adding an antibody prepared against pure Group A mucopeptide to the mononuclear cell. In preferred embodiments the mononuclear cell is human.

Another aspect of the present invention is derived on the premise that the mucopeptide complex elicits an immune response that is comparable to rheumatoid arthritis. This aspect of the invention includes methods of identifying an antigenic peptide. One specific embodiment of such a method comprises the steps of administering to an animal a selected peptide, then administering to the animal an amount of Group A mucopeptide capable of inducing experimental arthritis in said animal, and finally, identifying an antigenic peptide on the basis of the ability of the selected peptide to elicit a protective antibody response against the Group A mucopeptide induced experimental arthritis in the animal. In one specific embodiment of this type the animal is a rat and the amount of Group A mucopeptide administered is in a dose equivalent to the Group A cell fragments containing 15-30 μg of rhamnose per gram of body weight of the rat, *i.e.*, a dose equivalent to 3-6 mgs of rhamnose for a 200 gram rat. [The amount of mucopeptide is typically expressed as a function of the quantity of rhamnose determined on the mucopeptide, because the rhamnose content of the mucopeptide is relatively constant and readily determined]. In a preferred embodiment of this type, 125 μgs to 250 μgs of the selected peptide per gram of body weight of the rat, *i.e.*, a dose of 25 to 50 mgs of the selected peptide for a 200 gram rat, is administered to the animal prior to the administration of the Group A mucopeptide.

The selected peptide may be selected on the basis of its ability to bind to an anti-Group A mucopeptide antibody and inhibit its binding to the Group A mucopeptide. One related method for selecting such a peptide comprises contacting an assortment of random peptides with an anti-Group A mucopeptide antibody, and choosing a candidate peptide on the basis of the ability of the candidate peptide to bind the Group A mucopeptide antibody; contacting the candidate peptide with the Group A mucopeptide, and choosing the selected peptide on the basis of the ability of the candidate peptide not to bind the Group A mucopeptide. The selected peptide may then be recovered and/or otherwise identified and used in the screening assay described above for identifying an antigenic peptide of the present invention.

As for the earlier embodiments of the present invention the assortment of random peptides may be obtained from a phage library. In one specific embodiment the selected peptides are obtained by contacting a phage library with permissive bacteria. The phage library contains an assortment of phage with each individual phage comprising a random DNA sequence that encodes specific peptides. The DNA sequences of the phage library are expressed and a variety of random peptides are generated. The selected peptide may be recovered as follows: the phage encoding the selected peptide can be purified and the DNA that encodes the selected peptide can be sequenced. The amino acid sequence for the selected peptide can then be deduced from the DNA sequence. Alternatively, synthetic combinatorial libraries can be used.

The selected peptides can then be synthesized per their respective amino acid sequences. The synthesis can be performed, for example, by standard genetic engineering techniques or more preferably by solid phase peptide synthesis.

The antigenic peptides obtained by the methods described herein are also part of the present invention. These inhibitory peptides have the following characteristics: they do not bind to the Group A mucopeptide; they do bind to an anti-Group A mucopeptide antibody; and they elicit a protective antibody response in animals against Group A mucopeptide induced experimental arthritis. The present invention also includes pharmaceutical compositions comprising an antigenic peptide of the present invention and a pharmaceutically acceptable carrier.

The present invention also includes methods of analyzing the ability to prevent the onset of experimental arthritis in an animal induced to develop experimental arthritis. In one embodiment the method comprises administering to the animal an antibody prepared against the Group A mucopeptide prior to the onset of experimental arthritis, thereby preventing the onset of experimental arthritis. In another embodiment a method for preventing the onset of experimental arthritis comprises administering to the animal a pharmaceutical composition comprising an antigenic peptide of the present invention and a pharmaceutically acceptable carrier prior to the onset of experimental arthritis.

The present invention also includes methods of treating experimental arthritis in an animal. In one embodiment the method comprises administering to the animal an antibody prepared against the Group A mucopeptide after the onset of experimental arthritis, whereby the experimental arthritis in said animal is ameliorated. In a related embodiment, the present invention includes a method of administering to the animal the pharmaceutical composition comprising an antigenic peptide of the present invention and a pharmaceutically acceptable carrier after the onset of experimental arthritis, whereby the experimental arthritis in said animal is ameliorated.

It should be understood that variations in the amino acid sequence of the antigenic and inhibitory peptides can be made in order to increase their binding affinities to the anti-Group A mucopeptide antibody and the Group A mucopeptide respectively. For example, slight variations include replacing one acidic amino acid with another, such as replacing an aspartate with a glutamate. More dramatic modifications are also envisioned such as substituting an ornithine for an arginine or even a *d*-amino acid for its corresponding *l*-amino acid. All such modifications for optimizing the desired properties of these peptides are contemplated by the present invention.

These and other aspects of the present invention will be better appreciated by reference to the following drawings and Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1. PRIOR ART. The structure of the mucopeptide complex of the gram-positive bacterial cell wall. Depiction of a schematic drawing of the polysaccharide-peptide complex (mucopeptide complex) of the cell wall of gram positive bacteria.

FIGURE 2. PRIOR ART. The structure of the glycolipid complex of the gram-negative bacterial cell wall. Depiction of a schematic drawing of the glycolipid (Lipid A) of the cell wall of gram negative bacteria.

FIGURE 3. Comparison of the structures of the mucopeptide and the glycolipid complex. Depiction of the comparison of the structures of the peptidoglycan (mucopeptide complex) and Lipid A.

FIGURE 4. TNFα induction by bacterial cell wall components in the presence and absence of antibodies prepared against the pure group A streptococcal mucopeptide. A three-dimensional bar graph showing a comparison of the ng of TNF *alpha* produced in human mononuclear cells (y-axis) by a single addition of 600 ng of the mucopeptide complex (Figure 4A) or a single addition of 20 ng of LPS (Figure 4B) in the presence (stippled bar) or absence (shaded bar) of antibodies prepared against the pure Group A streptococcal mucopeptide.

DETAILED DESCRIPTION OF THE INVENTION

The present invention discloses novel methods and compositions for treating septic shock, Crohn's disease, psoriasis, and rheumatoid arthritis through the use of antibodies raised against the Group A mucopeptide. These antibodies may be used directly, or indirectly by identifying specific peptides that can be used in such treatments.

Whether or not the 70 kDa protein, discussed above, is the long sought after LPS receptor, the fact that the streptococcal mucopeptide complex can inhibit the interaction of the LPS and this 70 kDa protein is consistent with the LPS and the mucopeptide complex having some structural similarity. Accordingly, the present invention includes the use of antibodies prepared against the pure Group A streptococcal mucopeptide that are able to block the

induction of TNF *alpha* in human mononuclear cells regardless of whether they were stimulated by either Group A mucopeptide obtained from gram positive bacteria or LPS obtained from gram negative organisms (see Figure 4). As seen in Figure 3, if one looks at the three dimensional structures of both groups, there is a remarkable similarity in the backbone moieties. This similarity further suggest that a common structural element present in both Gram positive and Gram negative organisms is capable of binding to a receptor present on human mononuclear cells and inducing TNF alpha production.

The present invention describes a novel approach of using anti-mucopeptide antibodies to help identify novel peptides which will bind to the common structural element present on the Group A mucopeptide and on the LPS molecule. These peptides resemble the binding domains within the anti-mucopeptide antibodies and thus can block the binding of the cell receptor to the common structural element present in Group A mucopeptide and LPS. This, in turn, inhibits the induction of TNF *alpha* by the target cell. Accordingly, the present invention includes the investigation of diseases, such as septic shock, Crohn's disease, psoriasis and experimental arthritis (an animal model for Rheumatoid Arthritis) using this novel approach. It would be understood by any person with skill in the relevant art that such diseases are only meant to be exemplary and other diseases having related mechanisms can also be successfully treated by the teachings herein.

As used herein, the terms "mucopeptide complex", "Group A mucopeptide", "peptidoglycan", and the "polysaccharide-peptide complex" are used interchangeably and denote an integral aspect of the cell wall of gram positive bacteria. Pieces of this integral aspect induce an immunological response in mammals that can include septic shock.

As used herein, the terms "Lipid A", "glycolipid", "lipopolysaccharides" or "LPS" are used interchangeably and denote an integral aspect of the cell wall of gram negative bacteria. Pieces of this integral aspect induce an immunological response in mammals that can include septic shock.

As used herein an "inhibitory peptide" has the following characteristics: it binds the Group A mucopeptide; it binds the LPS molecule; and it inhibits an anti-Group A mucopeptide antibody from binding the Group A mucopeptide.

As used herein an "antigenic peptide" has the following characteristics: it does not bind to the Group A mucopeptide; but it does bind to an anti-Group A mucopeptide antibody; and it elicits a protective antibody response in animals against Group A mucopeptide induced experimental arthritis.

As used herein, a "common structural element" is used interchangeably with a "common structural motif" and denotes a three dimensional structural similarity which allows at least one binding partner to selectively bind to at least two otherwise distinctive molecules that contain such a common structural motif with a comparable affinity.

As used herein "experimental arthritis" has clinical symptoms including redness and swelling of the paws of the animal. Such symptoms can be monitored by observation and/or by measurement of paw diameter. These symptoms wax and wane over time. An increased paw size is indicative of arthritis.

Antibodies

According to the invention, mucopeptide prepared from natural sources, or produced recombinantly or by chemical synthesis, and fragments or other derivatives or analogs thereof, including fusion proteins, may be used as an immunogen to generate antibodies that recognize the mucopeptide and LPS. Similarly, LPS prepared from natural sources, or by chemical synthesis, and fragments or other derivatives or analogs thereof, may be used as an immunogen to generate antibodies that recognize both the mucopeptide and LPS. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library.

A molecule is "antigenic" when it is capable of specifically interacting with an antigen recognition molecule of the immune system, such as an immunoglobulin (antibody) or T cell antigen receptor. An antigenic polypeptide contains at least about 5, and preferably at least about 10, amino acids. An antigenic portion of a molecule can be that portion that is immunodominant for antibody or T cell receptor recognition, or it can be a portion used to generate an antibody to the molecule by conjugating the antigenic portion to a carrier molecule for immunization. A molecule that is antigenic need not be itself immunogenic, *i.e.*, capable of eliciting an immune response without a carrier.

An "antibody" is any immunoglobulin, including antibodies and fragments thereof, that binds a specific epitope. The term encompasses polyclonal, monoclonal, and chimeric antibodies, the last mentioned described in further detail in U.S. Patent Nos. 4,816,397 and 4,816,567, as well as antigen binding portions of antibodies, including Fab, $F(ab')_2$ and F(v) (including single chain antibodies). Accordingly, the phrase "antibody molecule" in its various grammatical forms as used herein contemplates both an intact immunoglobulin molecule and an immunologically active portion of an immunoglobulin molecule containing the antibody combining site. An "antibody combining site" is that structural portion of an antibody molecule comprised of heavy and light chain variable and hypervariable regions that specifically binds antigen.

Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contains the paratope, including those portions known in the art as Fab, Fab', $F(ab')_2$ and F(v), which portions are preferred for use in the therapeutic methods described herein.

Fab and $F(ab')_2$ portions of antibody molecules are prepared by the proteolytic reaction of papain and pepsin, respectively, on substantially intact antibody molecules by methods that are well-known. See for example, U.S. Patent No. 4,342,566 to Theofilopolous *et al.* Fab' antibody molecule portions are also well-known and are produced from $F(ab')_2$ portions followed by reduction of the disulfide bonds linking the two heavy chain portions as with mercaptoethanol, and followed by alkylation of the resulting protein mercaptan with a reagent such as iodoacetamide. An antibody containing intact antibody molecules is preferred herein.

The phrase "monoclonal antibody" in its various grammatical forms refers to an antibody having only one species of antibody combining site capable of immunoreacting with a particular antigen. A monoclonal antibody thus typically displays a single binding affinity for any antigen with which it immunoreacts. A monoclonal antibody may therefore contain an antibody molecule having a plurality of antibody combining sites, each immunospecific for a different antigen; e.g., a bispecific (chimeric) monoclonal antibody.

The term "adjuvant" refers to a compound or mixture that enhances the immune response to an antigen. An adjuvant can serve as a tissue depot that slowly releases the antigen and

also as a lymphoid system activator that non-specifically enhances the immune response [Hood *et al.*, in *Immunology*, p. 384, Second Ed., Benjamin/Cummings, Menlo Park, California (1984)]. Often, a primary challenge with an antigen alone, in the absence of an adjuvant, will fail to elicit a humoral or cellular immune response. Adjuvants include, but are not limited to, complete Freund's adjuvant, incomplete Freund's adjuvant, saponin, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil or hydrocarbon emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (*bacille Calmette-Guerin*) and *Corynebacterium parvum*. Preferably, the adjuvant is pharmaceutically acceptable.

Various procedures known in the art may be used for the production of polyclonal antibodies that cross-react with the mucopeptide and LPS, or fragment, derivative or analog thereof. For the production of antibody, various host animals can be immunized by injection with the mucopeptide or LPS, or a derivative (*e.g.*, fragment or fusion protein) thereof, including but not limited to rabbits, mice, rats, sheep, goats, etc. In one embodiment, the mucopeptide, LPS or fragment thereof can be conjugated to an immunogenic carrier, *e.g.*, bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (*bacille Calmette-Guerin*) and *Corynebacterium parvum*.

For preparation of monoclonal antibodies directed toward the mucopeptide, LPS or fragment, analog, or derivative thereof, any technique that provides for the production of antibody molecules by continuous cell lines in culture may be used. These include but are not limited to the hybridoma technique originally developed by Kohler *et al.*, *Nature*, 256:495-497 (1975), as well as the trioma technique, the human B-cell hybridoma technique [Kozbor *et al.*, *Immunology Today*, 4:72 (1983)], and the EBV-hybridoma technique to produce human monoclonal antibodies [Cole *et al.*, in *Monoclonal Antibodies and Cancer Therapy*, pp. 77-96, Alan R. Liss, Inc., (1985)]. Immortal, antibody-producing cell lines can be created by techniques other than fusion, such as direct transformation of B

lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. *See, e.g.*, M. Schreier *et al.*, "Hybridoma Techniques" (1980); Hammerling *et al.*, "Monoclonal Antibodies And T-cell Hybridomas" (1981); Kennett *et al.*, "Monoclonal Antibodies" (1980); *see also* U.S. Patent Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,451,570; 4,466,917; 4,472,500; 4,491,632; and 4,493,890.

In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing recent technology (PCT/US90/02545). According to the invention, human antibodies may be used and can be obtained by using human hybridomas [Cote *et al.*, *Proc. Natl. Acad. Sci. USA*, 80:2026-2030 (1983)] or by transforming human B cells with EBV virus *in vitro* (Cole *et al.*, 1985, *supra*). In fact, according to the invention, techniques developed for the production of "chimeric antibodies" [Morrison *et al.*, *J. Bacteriol.*, 159-870 (1984); Neuberger *et al.*, *Nature*, 312:604-608 (1984); Takeda *et al.*, *Nature*, 314:452-454 (1985)] by splicing the genes from a mouse antibody molecule specific for the mucopeptide or LPS together with genes from a human antibody molecule of appropriate biological activity can be used; such antibodies are within the scope of this invention. Such human or humanized chimeric antibodies are preferred for use in therapy of human diseases or disorders (described *infra*), since the human or humanized antibodies are much less likely than xenogenic antibodies to induce an immune response, in particular an allergic response, themselves.

According to the invention, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce mucopeptide/LPS-specific single chain antibodies. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries [Huse *et al.*, *Science*, 246:1275-1281 (1989)] to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for the mucopeptide and LPS shared epitope.

Antibody fragments which contain the idiotype of the antibody molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, *e.g.*, radioimmunoassay, ELISA (enzyme-linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), Western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention. For example, to select antibodies which recognize a specific epitope common to the mucopeptide and LPS, one may assay generated hybridomas for a product which binds to a mucopeptide or LPS fragment containing such an epitope.

The foregoing antibodies can be used in methods known in the art relating to the localization and activity of the mucopeptide or LPS *e.g.*, for Western blotting, imaging the mucopeptide or LPS *in situ*, measuring levels thereof in appropriate physiological samples, etc.

In a specific embodiment, antibodies that agonize or antagonize the activity of the mucopeptide and LPS can be generated. Such antibodies can be tested using the assays described *infra* for identifying ligands.

In a specific embodiment, antibodies are developed by immunizing rabbits with synthetic peptides predicted by the sequence of the mucopeptide. Synthetic peptides may be conjugated to a carrier such as KLH hemocyanin or BSA using carbodiimide and used in Freund's adjuvant to immunize rabbits. The expressed peptide may be prepared in quantity and used to immunize rabbits in Freund's adjuvant.

In another specific embodiment, the mucopeptide is used to immunize chickens, and the chicken anti-mucopeptide antibodies are recovered from egg yolk, *e.g.*, by affinity purification on a mucopeptide-column. The affinity purified antibodies can be selected by

subsequent purification on a LPS-column. Preferably, chickens used in immunization are kept under specific pathogen free (SPF) conditions.

In another embodiment, the mucopeptide is used to immunize rabbits, and the polyclonal antibodies are immunopurified on a mucopeptide-column and a LPS-column prior to further use. The purified antibodies are particularly useful for semi-quantitative assays. Panels of monoclonal antibodies produced against the mucopeptide or LPS can be screened for various properties; *i.e.*, isotype, epitope, affinity, etc. Of particular interest are monoclonal antibodies that neutralize the activity of the mucopeptide or LPS to bind to the TNF receptor. Such monoclonals can be readily identified in by the assays described herein. High affinity antibodies are particularly useful for this neutralization process.

Preferably, the anti-modulator antibody used in the diagnostic and therapeutic methods of this invention is an affinity-purified polyclonal antibody. More preferably, the antibody is a monoclonal antibody (mAb). In addition, it is preferable for the anti-mucopeptide or anti-LPS molecules used herein be in the form of Fab, Fab', $F(ab')_2$ or F(v) portions of whole antibody molecules.

Peptides

The term "peptide" is used in its broadest sense to refer to a compound of two or more subunit amino acids, amino acid analogs, or peptidomimetics. The subunits may be linked by peptide bonds. In another embodiment, the subunit may be linked by other the bonds, *e.g.*, ester, ether, etc. As used herein the term "amino acid" refers to either natural and/or unnatural or synthetic amino acids, including glycine and both the D or L optical isomers, and amino acid analogs and peptidomimetics.

Using the "phage method" [Scott and Smith, 1990, *Science* **249**:386-390 (1990); Cwirla, et al., *Proc. Natl. Acad. Sci.*, **87**:6378-6382 (1990); Devlin et al., *Science*, **249**:404-406 (1990)], very large libraries can be constructed (10^6-10^8 chemical entities). A second approach uses primarily chemical methods, of which the Geysen method [Geysen et al., *Molecular Immunology* **23**:709-715 (1986); Geysen et al. *J. Immunologic Method* **102**:259-274 (1987)] and the method of Fodor et al. [*Science* **251**:767-773 (1991)] are examples. Furka et al. [*14th International Congress of Biochemistry, Volume 5*, Abstract FR:013 (1988); Furka, *Int. J. Peptide Protein Res.* **37**:487-493 (1991)], Houghton [U.S. Patent No.

4,631,211, issued December 1986] and Rutter et al. [U.S. Patent No. 5,010,175, issued April 23, 1991] describe methods to produce a mixture of peptides that can be tested as described herein. Such peptides can be further refined through modifications by chemical synthesis as described below.

In another aspect, synthetic libraries [Needels et al., *Proc. Natl. Acad. Sci. USA* 90:10700-4 (1993); Ohlmeyer et al., *Proc. Natl. Acad. Sci. USA* 90:10922-10926 (1993); Lam et al., International Patent Publication No. WO 92/00252; Kocis et al., International Patent Publication No. WO 9428028, each of which is incorporated herein by reference in its entirety], and the like can be used to screen for the peptides of the present invention. Synthetic peptides, prepared using the well known techniques of solid phase, liquid phase, or peptide condensation techniques, or any combination thereof, can include natural and unnatural amino acids. Amino acids used for peptide synthesis may be standard Boc (N^{α}-amino protected N^{α}-t-butyloxycarbonyl) amino acid resin with the standard de-protecting, neutralization, coupling and wash protocols of the original solid phase procedure of Merrifield (1963, J. Am. Chem. Soc. 85:2149-2154), or the base-labile N^{α}-amino protected 9-fluorenylmethoxycarbonyl (Fmoc) amino acids first described by Carpino and Han (1972, J. Org. Chem. 37:3403-3409). Both Fmoc and Boc N^{α}-amino protected amino acids can be obtained from Fluka, Bachem, Advanced Chemtech, Sigma, Cambridge Research Biochemical, Bachem, or Peninsula Labs or other chemical companies familiar to those who practice this art. In addition, the method of the invention can be used with other N^{α}-protecting groups that are familiar to those skilled in this art. Solid phase peptide synthesis may be accomplished by techniques familiar to those in the art and provided, for example, in Stewart and Young, 1984, Solid Phase Synthesis, Second Edition, Pierce Chemical Co., Rockford, IL; Fields and Noble, 1990, Int. J. Pept. Protein Res. 35:161-214, or using automated synthesizers, such as sold by ABS. Thus, peptides of the invention may comprise D-amino acids, a combination of D- and L-amino acids, and various "designer" amino acids (*e.g.*, β-methyl amino acids, Cα-methyl amino acids, and Nα-methyl amino acids, etc.) to convey special properties. Synthetic amino acids include ornithine for lysine, fluorophenylalanine for phenylalanine, and norleucine for leucine or isoleucine. Additionally, by assigning specific amino acids at specific coupling steps, α-helices, β turns, β sheets, γ-turns, and cyclic peptides can be generated.

In one aspect of the invention, the peptides may comprise a special amino acid at the C-terminus which incorporates either a CO_2H or $CONH_2$ side chain to simulate a free glycine or a glycine-amide group. Another way to consider this special residue would be as a D or L amino acid analog with a side chain consisting of the linker or bond to the bead. In one embodiment, the pseudo-free C-terminal residue may be of the D or the L optical configuration; in another embodiment, a racemic mixture of D and L-isomers may be used. In an additional embodiment, pyroglutamate may be included as the N-terminal residue of the peptide.

In a further embodiment, subunits of peptides that confer useful chemical and structural properties will be chosen. For example, peptides comprising D-amino acids will be resistant to L-amino acid-specific proteases *in vivo*. In addition, the present invention envisions preparing peptides that have more well defined structural properties, and the use of peptidomimetics, and peptidomimetic bonds, such as ester bonds, to prepare peptides with novel properties. In another embodiment, a peptide may be generated that incorporates a reduced peptide bond, i.e., R_1-CH_2-NH-R_2, where R_1 and R_2 are amino acid residues or sequences. A reduced peptide bond may be introduced as a dipeptide subunit. Such a molecule would be resistant to peptide bond hydrolysis, *e.g.*, protease activity. Such peptides would provide ligands with unique function and activity, such as extended half-lives *in vivo* due to resistance to metabolic breakdown, or protease activity. Furthermore, it is well known that in certain systems constrained peptides show enhanced functional activity (Hruby, 1982, Life Sciences 31:189-199; Hruby et al., 1990, Biochem J. 268:249-262); the present invention provides a method to produce a constrained peptide that incorporates random sequences at all other positions.

1. *Constrained and cyclic peptides.*

A constrained, cyclic or rigidized peptide may be prepared synthetically, provided that in at least two positions in the sequence of the peptide an amino acid or amino acid analog is inserted that provides a chemical functional group capable of crosslinking to constrain, cyclise or rigidize the peptide after treatment to form the cross-link. Cyclization will be favored when a turn-inducing amino acid is incorporated. Examples of amino acids capable of crosslinking a peptide are cysteine to form disulfides, aspartic acid to form a lactone or a lactam, and a chelator such as γ-carboxyl-glutamic acid (Gla) (Bachem) to chelate a transition metal and form a cross-link. Protected γ-carboxyl glutamic acid may be prepared

by modifying the synthesis described by Zee-Cheng and Olson (1980, Biophys. Biochem. Res. Commun. 94:1128-1132). A peptide in which the peptide sequence comprises at least two amino acids capable of crosslinking may be treated, *e.g.*, by oxidation of cysteine residues to form a disulfide or addition of a metal ion to form a chelate, so as to cross-link the peptide and form a constrained, cyclic or rigidized peptide.

2. *Non-classical amino acids that induce conformational constraints.*

The following non-classical amino acids may be incorporated in the peptide in order to introduce particular conformational motifs: 1,2,3,4-tetrahydroisoquinoline-3-carboxylate (Kazmierski et al., 1991, J. Am. Chem. Soc. 113:2275-2283); (2S,3S)-methyl-phenylalanine, (2S,3R)-methyl-phenylalanine, (2R,3S)-methyl-phenylalanine and (2R,3R)-methyl-phenylalanine (Kazmierski and Hruby, 1991, Tetrahedron Lett.); 2-aminotetrahydronaphthalene-2-carboxylic acid (Landis, 1989, Ph.D. Thesis, University of Arizona); hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (Miyake et al., 1989, J. Takeda Res. Labs. 43:53-76); β-carboline (D and L) (Kazmierski, 1988, Ph.D. Thesis, University of Arizona); HIC (histidine isoquinoline carboxylic acid) (Zechel et al., 1991, Int. J. Pep. Protein Res. 43); and HIC (histidine cyclic urea) (Dharanipragada).

The following amino acid analogs and peptidomimetics may be incorporated into a peptide to induce or favor specific secondary structures: LL-Acp (LL-3-amino-2-propenidone-6-carboxylic acid), a β-turn inducing dipeptide analog (Kemp et al., 1985, J. Org. Chem. 50:5834-5838); β-sheet inducing analogs (Kemp et al., 1988, Tetrahedron Lett. 29:5081-5082); β-turn inducing analogs (Kemp et al., 1988, Tetrahedron Lett. 29:5057-5060); ∝-helix inducing analogs (Kemp et al., 1988, Tetrahedron Lett. 29:4935-4938); γ-turn inducing analogs (Kemp et al., 1989, J. Org. Chem. 54:109:115); and analogs provided by the following references: Nagai and Sato, 1985, Tetrahedron Lett. 26:647-650; DiMaio et al., 1989, J. Chem. Soc. Perkin Trans. p. 1687; also a Gly-Ala turn analog (Kahn et al., 1989, Tetrahedron Lett. 30:2317); amide bond isostere (Jones et al., 1988, Tetrahedron Lett. 29:3853-3856); tretrazol (Zabrocki et al., 1988, J. Am. Chem. Soc. 110:5875-5880); DTC (Samanen et al., 1990, Int. J. Protein Pep. Res. 35:501:509); and analogs taught in Olson et al., 1990, J. Am. Chem. Sci. 112:323-333 and Garvey et al., 1990, J. Org. Chem. 56:436. Conformationally restricted mimetics of beta turns and beta bulges, and peptides containing them, are described in U.S. Patent No. 5,440,013, issued August 8, 1995 to Kahn.

3. *Derivatized and modified peptides.*

The present invention further provides for modification or derivatization of a peptide of the invention. Modifications of peptides are well known to one of ordinary skill, and include phosphorylation, carboxymethylation, and acylation. Modifications may be effected by chemical or enzymatic means.

In another aspect, glycosylated or fatty acylated peptide derivatives may be prepared. Preparation of glycosylated or fatty acylated peptides is well known in the art as exemplified by the following references:

1. Garg and Jeanloz, 1985, in Advances in Carbohydrate Chemistry and Biochemistry, Vol. 43, Academic Press.

2. Kunz, 1987, in Ang. Chem. Int. Ed. English 26:294-308.

3. Horvat et al., 1988, Int. J. Pept. Protein Res. 31:499-507.

4. Bardaji et al., 1990, Ang. Chem. Int. Ed. English, 23:231.

5. Toth et al., 1990, in Peptides: Chemistry, Structure and Biology, Rivier and Marshal, eds., ESCOM Publ., Leiden, pp. 1078-1079.

6. Torres et al., 1989, Experientia 45:574-576.

7. Torres et al., 1989, EMBO J. 8:2925-2932.

8. Hordever and Musiol, 1990, in Peptides: Chemistry, Structure and Biology, loc. cit., pp. 811-812.

9. Zee-Cheng and Olson, 1989, Biochem. Biophys. Res. Commun. 94:1128-1132.

10. Marki et al., 1977, Helv. Chem. Acta., 60:807.

11. Fuju et al. 1987, J. Chem. Soc. Chem. Commun., pp. 163-164.

12. Ponsati et al., 1990, Peptides 1990, Giralt and Andreu, eds., ESCOM Publ., pp. 238-240.

13. Fuji et al., 1987, 1988, Peptides: Chemistry and Biology, Marshall, ed., ESCOM Publ., Leiden, pp. 217-219.

There are two major classes of peptide-carbohydrate linkages. First, ether bonds join the serine or threonine hydroxyl to a hydroxyl of the sugar. Second, amide bonds join glutamate or aspartate carboxyl groups to an amino group on the sugar. In particular,

references 1 and 2, *supra*, teach methods of preparing peptide-carbohydrate ethers and amides. Acetal and ketal bonds may also bind carbohydrate to peptide.

Fatty acyl peptide derivatives may also be prepared. For example, and not by way of limitation, a free amino group (N-terminal or lysyl) may be acylated, *e.g.*, myristoylated. In another embodiment an amino acid comprising an aliphatic side chain of the structure -$(CH_2)_nCH_3$ may be incorporated in the peptide. This and other peptide-fatty acid conjugates suitable for use in the present invention are disclosed in U.K. Patent GB-8809162.4, International Patent Application PCT/AU89/00166, and reference 5, *supra*.

Genetic Engineering of the Peptides

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual,* Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (herein "Sambrook et al., 1989"); *DNA Cloning: A Practical Approach,* Volumes I and II (D.N. Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed. 1984); *Nucleic Acid Hybridization* [B.D. Hames & S.J. Higgins eds. (1985)]; *Transcription And Translation* [B.D. Hames & S.J. Higgins, eds. (1984)]; *Animal Cell Culture* [R.I. Freshney, ed. (1986)]; *Immobilized Cells And Enzymes* [IRL Press, (1986)]; B. Perbal, *A Practical Guide To Molecular Cloning* (1984); F.M. Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (1994).

The term "sequence similarity" in all its grammatical forms refers to the degree of identity or correspondence between nucleic acid or amino acid sequences of proteins that do not share a common evolutionary origin (*see* Reeck et al., *supra*). However, in common usage and in the instant application, the term "homologous," when modified with an adverb such as "highly," refers to sequence similarity and not a common evolutionary origin.

In a specific embodiment, two DNA sequences are "substantially homologous" or "substantially similar" when at least about 50% (preferably at least about 75%, and most preferably at least about 90 or 95%) of the nucleotides match over the defined length of the DNA sequences.

Similarly, in a particular embodiment, two amino acid sequences are "substantially homologous" or "substantially similar" when greater than 50% of the amino acids are identical, or greater than about 80% are similar (functionally identical). Preferably, the similar or homologous sequences are identified by alignment using, for example, the GCG (Genetics Computer Group, Program Manual for the GCG Package, *Version 7*, Madison, Wisconsin) pileup program.

The term "corresponding to" is used herein to refer similar or homologous sequences, whether the exact position is identical or different from the molecule to which the similarity or homology is measured. Thus, the term "corresponding to" refers to the sequence similarity, and not the numbering of the amino acid residues or nucleotide bases.

Identification of a specific DNA encoding a particularly long peptide of the present invention may be accomplished in a number of ways. For example, if an amount of a portion of the DNA or a fragment thereof, is available and can be purified and labeled, the generated DNA fragments may be screened by nucleic acid hybridization to a labeled probe (Benton and Davis, 1977, Science 196:180; Grunstein and Hogness, 1975, Proc. Natl. Acad. Sci. U.S.A. 72:3961). For example, a set of oligonucleotides corresponding to the amino acid sequence of the peptide can be prepared and used as probes for DNA. Those DNA fragments with substantial homology to the probe will hybridize. The greater the degree of homology, the more stringent hybridization conditions can be used.

Expression of Antigenic and Inhibitory Peptides and Their Analogs

The nucleotide sequence coding for a desired peptide, derivative or analog thereof, or a functionally active derivative, can be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted peptide-coding sequence. Such elements are termed herein a "promoter." Thus, the nucleic acid encoding a peptide of the invention is operationally associated with a promoter in an expression vector of the invention. An expression vector also preferably includes a replication origin.

The necessary transcriptional and translational signals can be provided on a recombinant expression vector.

Potential host-vector systems include but are not limited to mammalian cell systems infected with virus (*e.g.*, vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (*e.g.*, baculovirus); microorganisms such as yeast containing yeast vectors; or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

The cell into which the vector containing the *peptide* DNA is cultured in an appropriate cell culture medium under conditions that provide for expression of the peptide by the cell.

Any of a number of methods for the insertion of DNA fragments into a such a vector may be used to construct expression vectors containing a nucleic acid consisting of appropriate transcriptional/translational control signals and the peptide coding sequences. These methods may include *in vitro* DNA and synthetic techniques.

Expression of peptide may be controlled by any promoter/enhancer element known in the art, but these regulatory elements must be functional in the host selected for expression. Promoters which may be used to control the peptide nucleic acid expression include, but are not limited to, the SV40 early promoter region (Benoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the *tac* promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25); see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter; and the animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals.

Expression vectors containing a nucleic acid encoding an antigenic or inhibitory peptide of the invention can be identified by four general approaches: (a) PCR amplification of the

desired plasmid DNA or specific mRNA, (b) nucleic acid hybridization, (c) presence or absence of selection marker gene functions, and (d) expression of inserted sequences. In the first approach, the nucleic acids can be amplified by PCR to provide for detection of the amplified product. In the second approach, the presence of a foreign gene inserted in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to an inserted marker gene. In the third approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "selection marker" gene functions (*e.g.*, β-galactosidase activity, thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign genes in the vector. In another example, if the nucleic acid encoding the peptide is inserted within the "selection marker" gene sequence of the vector, recombinants containing the peptide insert can be identified by the absence of the selection marker gene function. In the fourth approach, recombinant expression vectors can be identified by assaying for the activity, biochemical, or immunological characteristics of the peptide expressed by the recombinant, by the methods described herein.

A wide variety of host/expression vector combinations may be employed in expressing the DNA sequences of this invention. Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences. Suitable vectors include derivatives of SV40 and known bacterial plasmids, e.g., *E. coli* plasmids col El, pCR1, pBR322, pMal-C2, pET, pGEX (Smith *et al.*, 1988, Gene 67:31-40), pMB9 and their derivatives, plasmids such as RP4; phage DNAS, e.g., the numerous derivatives of phage λ, e.g., NM989, and other phage DNA, e.g., M13 and filamentous single stranded phage DNA; yeast plasmids such as the 2μ plasmid or derivatives thereof; vectors useful in eukaryotic cells, such as vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and phage DNAs, such as plasmids that have been modified to employ phage DNA or other expression control sequences; and the like.

For example, in a baculovirus expression systems, both non-fusion transfer vectors, such as but not limited to pVL941 (*Bam*H1 cloning site; Summers), pVL1393 (*Bam*H1, *Sma*I, *Xba*I, *Eco*R1, *Not*I, *Xma*III, *Bgl*II, and *Pst*I cloning site; Invitrogen), pVL1392 (*Bgl*II, *Pst*I, *Not*I, *Xma*III, *Eco*RI, *Xba*I, *Sma*I, and *Bam*H1 cloning site; Summers and Invitrogen), and pBlue*Bac*III (*Bam*H1, *Bgl*II, *Pst*I, *Nco*I, and *Hind*III cloning site, with blue/white

recombinant screening possible; Invitrogen), and fusion transfer vectors, such as but not limited to pAc700 (*Bam*H1 and *Kpn*I cloning site, in which the *Bam*H1 recognition site begins with the initiation codon; Summers), pAc701 and pAc702 (same as pAc700, with different reading frames), pAc360 (*Bam*H1 cloning site 36 base pairs downstream of a polyhedron initiation codon; Invitrogen(195)), and pBlueBacHisA, B, C (three different reading frames, with *Bam*H1, *Bgl*II, *Pst*I, *Nco*I, and *Hind*III cloning site, an N-terminal peptide for ProBond purification, and blue/white recombinant screening of plaques; Invitrogen (220)) can be used.

Once a particular synthetic DNA molecule is identified and isolated, several methods known in the art may be used to propagate it. Once a suitable host system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As previously explained, the expression vectors which can be used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors (*e.g.*, lambda), and plasmid and cosmid DNA vectors, to name but a few.

Vectors are introduced into the desired host cells by methods known in the art, *e.g.*, transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), use of a gene gun, or a DNA vector transporter (see, *e.g.*, Wu et al., 1992, J. Biol. Chem. 267:963-967; Wu and Wu, 1988, J. Biol. Chem. 263:14621-14624; Hartmut et al., Canadian Patent Application No. 2,012,311, filed March 15, 1990).

Administration

According to the invention, the component or components of a therapeutic composition of the invention may be introduced parenterally, transmucosally, *e.g.*, orally, nasally, or rectally, or transdermally. Preferably, administration is parenteral, *e.g.*, via intravenous injection, and also including, but is not limited to, intra-arteriole, intramuscular, intradermal, subcutaneous, intraperitoneal, intraventricular, and intracranial administration.

In another embodiment, the therapeutic compound can be delivered in a vesicle, in particular a liposome [*see* Langer, *Science* 249:1527-1533 (1990); Treat et al., in

Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss: New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*]. To reduce its systemic side effects, this may be a preferred method for introducing the peptides and antibodies of the present invention.

In yet another embodiment, the therapeutic compound can be delivered in a controlled release system. For example, the peptides may be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump may be used [*see* Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* **14**:201 (1987); Buchwald et al., *Surgery* **88**:507 (1980); Saudek et al., *N. Engl. J. Med.* **321**:574 (1989)]. In another embodiment, polymeric materials can be used [*see Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Press: Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley: New York (1984); Ranger and Peppas, *J. Macromol. Sci. Rev. Macromol. Chem.* **23**:61 (1983); see also Levy et al., *Science* **228**:190 (1985); During et al., *Ann. Neurol.* **25**:351 (1989); Howard et al., *J. Neurosurg.* **71**:105 (1989)]. In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, *i.e.*, the brain, thus requiring only a fraction of the systemic dose [*see, e.g.*, Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)].

Other controlled release systems are discussed in the review by Langer [*Science* **249**:1527-1533 (1990)].

In a further aspect, recombinant cells that have been transformed with nucleic acids encoding either the antigenic or inhibitory peptides of the present invention and express high levels of these peptides can be transplanted in a subject in need of these peptides. Preferably autologous cells transformed with these peptides are transplanted to avoid rejection; alternatively, technology is available to shield non-autologous cells that produce soluble factors within a polymer matrix that prevents immune recognition and rejection.

Thus, the therapeutic peptides of the present invention can be delivered by intravenous, intraarterial, intraperitoneal, intramuscular, or subcutaneous routes of administration. Alternatively, these peptides properly formulated, can be administered by nasal or oral

administration. A constant supply of these peptides can be ensured by providing a therapeutically effective dose (*i.e.*, a dose effective to induce metabolic changes in a subject) at the necessary intervals, *e.g.*, daily, every 12 hours, etc. These parameters will depend on the severity of the disease condition being treated, other actions, the weight, age, and sex of the subject, and other criteria, which can be readily determined according to standard good medical practice by those of skill in the art.

A subject in whom administration of these peptides is an effective therapeutic regiment is preferably a human, but can be any animal. Thus, as can be readily appreciated by one of ordinary skill in the art, the methods and pharmaceutical compositions of the present invention are particularly suited to administration to any animal, particularly a mammal, and including, but by no means limited to, domestic animals, such as feline or canine subjects, farm animals, such as but not limited to bovine, equine, caprine, ovine, and porcine subjects, wild animals (whether in the wild or in a zoological garden), research animals, such as mice, rats, rabbits, goats, sheep, pigs, dogs, cats, etc., avian species, such as chickens, turkeys, songbirds, etc., *i.e.*, for veterinary medical use.

Nasal Delivery. Nasal delivery of the pharmaceutical compositions containing the inhibitory or antigenic peptides (or derivative thereof) is also contemplated. Nasal delivery allows the passage of the peptide to the blood stream directly after administering the therapeutic product to the nose, without the necessity for deposition of the product in the lung. Formulations for nasal delivery include those with dextran or cyclodextran.

For nasal administration, a useful device is a small, hard bottle to which a metered dose sprayer is attached. In one embodiment, the metered dose is delivered by drawing the solution of peptides into a chamber of defined volume, which chamber has an aperture dimensioned to aerosolize and aerosol formulation by forming a spray when a liquid in the chamber is compressed. The chamber is compressed to administer the peptides. In a specific embodiment, the chamber is a piston arrangement. Such devices are commercially available.

Alternatively, a plastic squeeze bottle with an aperture or opening dimensioned to aerosolize an aerosol formulation by forming a spray when squeezed. The opening is usually found in the top of the bottle, and the top is generally tapered to partially fit in the

nasal passages for efficient administration of the aerosol formulation. Preferably, the nasal inhaler will provide a metered amount of the aerosol formulation, for administration of a measured dose of the drug.

The term "mucosal penetration enhancer" refers to a reagent that increases the rate or facility of transmucosal penetration of ketamine, such as but not limited to, a bile salt, fatty acid, surfactant or alcohol. In specific embodiments, the permeation enhancer can be sodium cholate, sodium dodecyl sulphate, sodium deoxycholate, taurodeoxycholate, sodium glycocholate, dimethylsulfoxide or ethanol. Suitable penetration enhancers also include glycyrrhetinic acid (U.S. Patent No. 5,112,804 to Kowarski) and polysorbate-80, the latter preferably in combination with an non-ionic surfactant such as nonoxynol-9, laureth-9, poloxamer-124, octoxynol-9, or lauramide-DEA (European Patent EP 0 242 643 B1 by Stoltz).

Various and numerous methods are known in the art for transdermal administration of a drug, *e.g.*, via a transdermal patch. Transdermal patches are described in for example, U.S. Patent No. 5,407,713, issued April 18, 1995 to Rolando et al.; U.S. Patent No. 5,352,456, issued October 4, 1004 to Fallon et al.; U.S. Patent No. 5,332,213 issued August 9, 1994 to D'Angelo et al.; U.S. Patent No. 5,336,168, issued August 9, 1994 to Sibalis; U.S. Patent No. 5,290,561, issued March 1, 1994 to Farhadieh et al.; U.S. Patent No. 5,254,346, issued October 19, 1993 to Tucker et al.; U.S. Patent No. 5,164,189, issued November 17, 1992 to Berger et al.; U.S. Patent No. 5,163,899, issued November 17, 1992 to Sibalis; U.S. Patent Nos. 5,088,977 and 5,087,240, both issued February 18, 1992 to Sibalis; U.S. Patent No. 5,008,110, issued April 16, 1991 to Benecke et al.; and U.S. Patent No. 4,921,475, issued May 1, 1990 to Sibalis, the disclosure of each of which is incorporated herein by reference in its entirety.

It can be readily appreciated that a transdermal route of administration may be enhanced by use of a dermal penetration enhancer, *e.g.*, such as enhancers described in U.S. Patent No. 5,164,189 (*supra*), U.S. Patent No. 5,008,110 (*supra*), and U.S. Patent No. 4,879,119, issued November 7, 1989 to Aruga et al., the disclosure of each of which is incorporated herein by reference in its entirety.

The present invention may be better understood by reference to the following non-limiting Examples, which are provided as exemplary of the invention.

EXAMPLES

MATERIALS AND METHODS

Protocol for affinity purified anti-mucopeptide antibodies.

The anti-mucopeptide antibody was affinity purified in the following manner. Five ml of rabbit anti-mucopeptide antibody was first passed over a protein A sepharose column and the antibody fraction binding to the column was eluted in a 0.05 M sodium Acetate buffer pH 2.5. The volume of the eluant was adjusted back to the original volume of the serum (5 ml) and the pH was adjusted to pH 7.2 with 5N NAOH. 5 mg of streptococcal mucopeptide (dry weight) was then mixed with 0.5 ml of anti-mucopeptide purified antibody and then rotated gently at 37°C for 2-3 hours. The sample was stored overnight in the cold at 4°C. The following day, the sample was centrifuged at 14000 RPM for 15-20 minutes. The supernatant was removed and the pellet was resuspended in 0.5 ml of anti-mucopeptide purified antibody and the incubation procedure was repeated. This procedure was repeated once more for the third time.

Following these absorptions, the pellet was resuspended in 1 ml of 0.05 M sodium acetate buffer pH 2.5 and rotated gently at room temperature for 1 hour and then centrifuged as described above. The pH of the supernatant was brought back to pH 7.2 by the addition of 5N NAOH and then tested with an ELISA assay using a preparation of sonicated mucopeptide at a concentration of 1ug/well. The ELISA titer was compared to the original non-absorbed antibody and the volume adjusted so that the absorbed antibody has the same titer as the original non-absorbed anti-mucopeptide antibody.

The biological activity of the affinity purified antibody was then tested using human mononuclear cell preparations as described above. Serial dilutions of the affinity purified anti-mucopeptide antibody was then added to the wells followed by the addition of different concentrations of LPS and streptococcal cell wall mucopeptide preparations starting at a concentration of 100 μg/ml. Control samples included cells in which the antibody wasn't added. The cell suspension was incubated for 24 hrs at 37°C in a CO_2 incubator and then

following the subsequent centrifugation, the supernatants were harvested and tested for TNF *alpha* production. As an additional control phytohemagglutinin-stimulated cells were tested to rule out the possibility that non-specific inhibitors were contained in the affinity purified antibody preparation. The TNF *alpha* assays were carried out with a standard capture ELISA kit from Endogen (Cambridge, MA).

EXAMPLE 1

Identifying peptides useful for the treatment of septic shock.

The observation that the anti-mucopeptide antibody blocks the induction of TNF production by both mucopeptide and LPS suggests that there is a common epitope in both structures. This, in turn, further suggests that binding domains on some anti-mucopeptide antibodies have the right sequence to bind to both of these compounds. These binding sequences are located in the following manner. Phage libraries have been constructed which when infected into host *E. coli* produce random peptide sequences of approximately 10 to 15 amino acids [Parmley and Smith, Gene 73:305-318 (1988), Scott and Smith, Science 249:386-249 (1990)]. Specifically, the phage library can be mixed in low dilutions with permissive *E. coli* in low melting point LB agar which is then poured on top of LB agar plates. After incubating the plates at 37°C for a period of time, small clear plaques in a lawn of *E. coli* will form which represents active phage growth and lysis of the *E. coli*. A representative of these phages can be absorbed to nylon filters by placing dry filters onto the agar plates. The filters can be marked for orientation, removed, and placed in washing solutions to block any remaining absorbent sites. The filters can then be placed in a solution containing radioactive mucopeptide complex. After a specified incubation period, the filters can be thoroughly washed and developed for autoradiography. Plagues containing the phage that bind to the mucopeptide complex can be identified. These phages can be further cloned and then retested for their ability to bind to the mucopeptide complex as before. Once the phages have been purified, the binding sequence contained within the phage can be determined by standard DNA sequencing techniques. Once the DNA sequence is known, synthetic peptides can be generated which represents these sequences. These peptides can be tested for their ability to: (1) bind the mucopeptide complex, (2) bind to lipid A, and (3) inhibit the anti-mucopeptide antibodies.

A single peptide with strong positive results indicates a single dominant binding peptide. Isolation of multiple peptides indicates that the peptides have a common sequence, e.g. the phage library has generated the exact same peptide more than once, or several difference peptides have a similar reactivity.

The peptide(s) shown positive by ELISA, can be synthesized and assayed to test whether it (they) can block the anti-mucopeptide effect on TNF induction by either LPS or mucopeptide. The effective peptide(s) can be synthesized in large quantities for use in animal models of shock and eventually in humans to prevent septic shock. It should be emphasized that synthetic peptide production is relatively non-labor intensive, easily manufactured, quality controlled and thus, large quantities of the desired product can be produced quite cheaply. Similar combinations of mass produced synthetic peptides have recently been used in trials of a malaria vaccine with great success [Patarroyo, Vaccine 10:175-178 (1990)] and shown to be quite safe for use in humans.

EXAMPLE 2

The use of anti-Group A mucopeptide antibodies and peptides in the treatment of experimental arthritis.

The disease rheumatoid arthritis is a disease of unknown etiology but presumed to be an autoimmune disease involving the target organ, the joints. While many experimental models of arthritis have been proposed (see Table 1), the streptococcal induced model is preferred for the following reasons. First, it is a single dose of a sonicated cell wall preparation which, unlike the other models, induces arthritis which then waxes and wanes for over half the life of the animal. Secondly, the end result bears a striking resemblance to the human disease. Finally, it is cell mediated and the model can be transferred to naive recipients by passive administration of donor lymphocytes without the streptococcal antigen being present. TNF plays an important role in disease induction and in an experimental model the disease can be blocked by injecting soluble TNF receptor molecules just after initiation of the disease.

Table 1

Features of Rheumatoid Arthritis and Animal Models of Arthritis

	Rheumatoid Arthritis	Streptococcal Arthritis	Adjuvant Arthritis	Collagen Arthritis
Clinical Features				
Recurrent	+	+	-	-
Joint Distribution	+	+	+	+
Nodules	+	+	-	-
Pathological Features				
Monocyte Infiltrates	+	+	+	+
Pannus	+	+	+	+/-
Cellular Features				
Ia Expression	+	+	ND	+
Serological Features				
Rheumatoid Factor	+	?	-	ND
Complement Deposition	+	+	ND	ND
Anti II Collagen	+	?	+	ND

ND indicates that it has not been determined.

Since the inciting agent is primarily the streptococcal mucopeptide-polysaccharide complex, the approach to the model proceeds by two routes of investigation. The first approach induces the experimental arthritis in rats. At selected times, either before or after the onset of arthritis, animals are injected with the anti-mucopeptide antibody. If the injection is before the onset of the disease, the anti-mucopeptide antibody protects against disease induction. If injected after the onset of disease, it ameliorates the symptoms and severity of disease. Since the antibody can confer passive protection, the peptides described above can prevent the onset of the induced arthritis when injected concurrently with the mucopeptide complex by I.V. or I.P.

A second approach is based on a interesting observation made previously in the model [Janusz *et al.*, J.Exp.Med. **160**:1360-1366 (1984)]. If one enzymatically digests the mucopeptide-polysaccharide complex with enzymes (phage lysin, mutanolysin, etc.) into

smaller pieces and injects these fragments, the recipient animal, after a period of time, is no longer susceptible to the induction of arthritis by the mucopeptide complex. Thus, these injected fragments seem to protect against induction of classical arthritis by the larger complex. This protection can be mediated through eliciting anti-mucopeptide antibodies directed against the responsible epitope which induces the arthritis.

These experiments can be extended to show that this protection can be passively transferred to a naive animal by antibodies, either by injecting the available anti-mucopeptide antibodies or those antibodies elicited after injecting enzymatically derived fragments of the mucopeptide complex. After determining that protection from induced arthritic disease can be mediated through antibodies, these antibodies can be used to identify peptide mimitopes of the mucopeptide fragments which will elicit a similar protective antibody response. Using the random peptide phage libraries discussed herein, the protective antibodies can be used to identify peptides which mimic the structure of the fragments of the mucopeptide complex. These peptides have the following characteristics: (1) they react with the anti-mucopeptide antibodies, (2) they do not bind to the mucopeptide complex, and (3) they do not elicit a protective antibody response in animals against mucopeptide complex induced arthritic disease. A similar strategy has been successfully used to find peptides that mimic the structure of Group C polysaccharide capsular antigens of *Neisseria mengitidis* [Westerink and Giardina, Microb.Pathog. 12:19-26 (1992)]. These peptides can be used to induce a protective antibody response to experimental arthritis.

The various forms of human arthritis (Crohn's disease, psoriatic arthritis, etc.) all appear to have a common antigen which is driving the etiology. The identification of a common epitope or mimitope to all these antigens, can lead to the amelioration or prevention of the manifestations of a number of these arthrides.

The present invention is not to be limited in scope by the specific embodiments describe herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description.

All documents cited above, are herein incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. A method of identifying an inhibitory peptide comprising contacting a selected peptide with a Group A mucopeptide and an anti-Group A mucopeptide antibody wherein an inhibitory peptide is selected on the basis of the ability of the selected peptide to inhibit the binding of the anti-Group A mucopeptide antibody to the Group A mucopeptide;

wherein the selected peptide is able to bind to both the Group A mucopeptide and an LPS molecule.

2. The method of Claim 1 wherein the selected peptide binds to a common structural element present in the Group A mucopeptide and the LPS molecule.

3. The method of Claim 2 wherein the selected peptide is selected by a method comprising:

(a) contacting an assortment of random peptides with the Group A mucopeptide, wherein a candidate peptide is chosen from the assortment of random peptides on the basis of the ability of the candidate peptide to bind to the Group A mucopeptide; and

(b) contacting the candidate peptide with the LPS molecule, wherein a selected peptide is chosen on the basis of the ability of the candidate peptide to bind to the LPS molecule.

4. The method of Claim 3 wherein the assortment of random peptides is obtained from a phage library.

5. The method of Claim 4 further comprising recovering the inhibitory peptide which comprises the steps of:

A. purifying the phage encoding the inhibitory peptide;

B. sequencing the DNA sequence that encodes the inhibitory peptide contained in the purified phage, whereby an amino acid sequence for the inhibitory peptide can be deduced; and

C. chemically synthesizing the inhibitory peptide according to the amino acid sequence.

6. An inhibitory peptide obtained by the method of Claim 1 having the following characteristics:

(i) it binds the Group A mucopeptide;

(ii) it binds the LPS molecule; and

(iii) it inhibits an anti-Group A mucopeptide antibody.

7. The inhibitory peptide of Claim 6 which further inhibits the bacterial-mediated production of tumor necrosis factor in a target cell.

8. The inhibitory peptide of Claim 7 wherein the target cell is a mononuclear cell.

9. A pharmaceutical composition for treating septic shock comprising the inhibitory peptide of Claim 8 and a pharmaceutically acceptable carrier.

10. A method of treating septic shock in mammals, comprising administering to a mammal a therapeutically effective amount of the pharmaceutical composition of Claim 9.

11. A pharmaceutical composition for treating experimental arthritis in an animal comprising the inhibitory peptide of Claim 8 and a pharmaceutically acceptable carrier.

12. A method of blocking the induction of TNFα in a mononuclear cell, comprising adding an antibody prepared against pure Group A mucopeptide to the mononuclear cell, wherein said induction is stimulated by the LPS molecule.

13. The method of Claim 12 wherein said mononuclear cell is human.

14. A method of preventing the onset of arthritis in an animal comprising administering an antibody prepared against Group A mucopeptide to said animal prior to the onset of arthritis, whereby the onset of arthritis in said animal is prevented.

15. A method of treating arthritis in an animal suffering from arthritis comprising administering the pharmaceutical composition of Claim 11 to said animal after the onset of arthritis, whereby the arthritis in said animal is ameliorated.

16. A method of identifying an antigenic peptide comprising:

(a) administering a selected peptide to an animal;

(b) administering an amount of Group A mucopeptide to said animal, wherein said amount of Group A mucopeptide is capable of inducing experimental arthritis in said animal; and

(c) identifying an antigenic peptide on the basis of the ability of the selected peptide to elicit a protective antibody response against the Group A mucopeptide induced experimental arthritis in said animal; wherein the selected peptide is selected on the basis of its ability to bind to an anti-Group A mucopeptide antibody and its inability to bind to the Group A mucopeptide.

17. The method of Claim 16 wherein the selected peptide is selected by a method comprising

A. contacting an assortment of random peptides with an anti-Group A mucopeptide antibody, wherein a candidate peptide is chosen from the assortment of random peptides on the basis of the ability of the candidate peptide to bind the Group A mucopeptide antibody;

B. contacting the candidate peptide with the Group A mucopeptide, wherein a selected peptide is chosen on the basis of the ability of the candidate peptide not to bind the Group A mucopeptide; and

C. recovering the selected peptide.

18. The method of Claim 17 wherein the assortment of random peptides is obtained from a phage library.

19. The method of Claim 18 wherein the step of recovering the selected peptide comprises:

(i) purifying the phage encoding the selected peptide;

(ii) sequencing the DNA sequence that encodes the selected peptide contained in the purified phage, whereby an amino acid sequence for the selected peptide can be deduced; and

(iii) chemically synthesizing the selected peptide according to the amino acid sequence.

20. An antigenic peptide obtained by the method of Claim 16 having the following characteristics:

(i) it does not bind to the Group A mucopeptide;

(ii) it binds to an anti-Group A mucopeptide antibody; and

(iii) it elicits a protective antibody response in animals against Group A mucopeptide induced experimental arthritis.

21. A pharmaceutical composition for treating experimental arthritis comprising the antigenic peptide of Claim 20 and a pharmaceutically acceptable carrier.

22. A method of preventing the onset of experimental arthritis in an animal induced to develop experimental arthritis comprising administering the pharmaceutical composition of Claim 21 to said animal prior to the onset of experimental arthritis, whereby the onset of experimental arthritis in said animal is prevented.

23. A method of ameliorating the effects of experimental arthritis in an animal induced to have experimental arthritis comprising administering the pharmaceutical composition of Claim 21 to said animal after the onset of experimental arthritis, whereby the effects of experimental arthritis in said animal is ameliorated.

600-1-171 (Sheet 1 of 4)

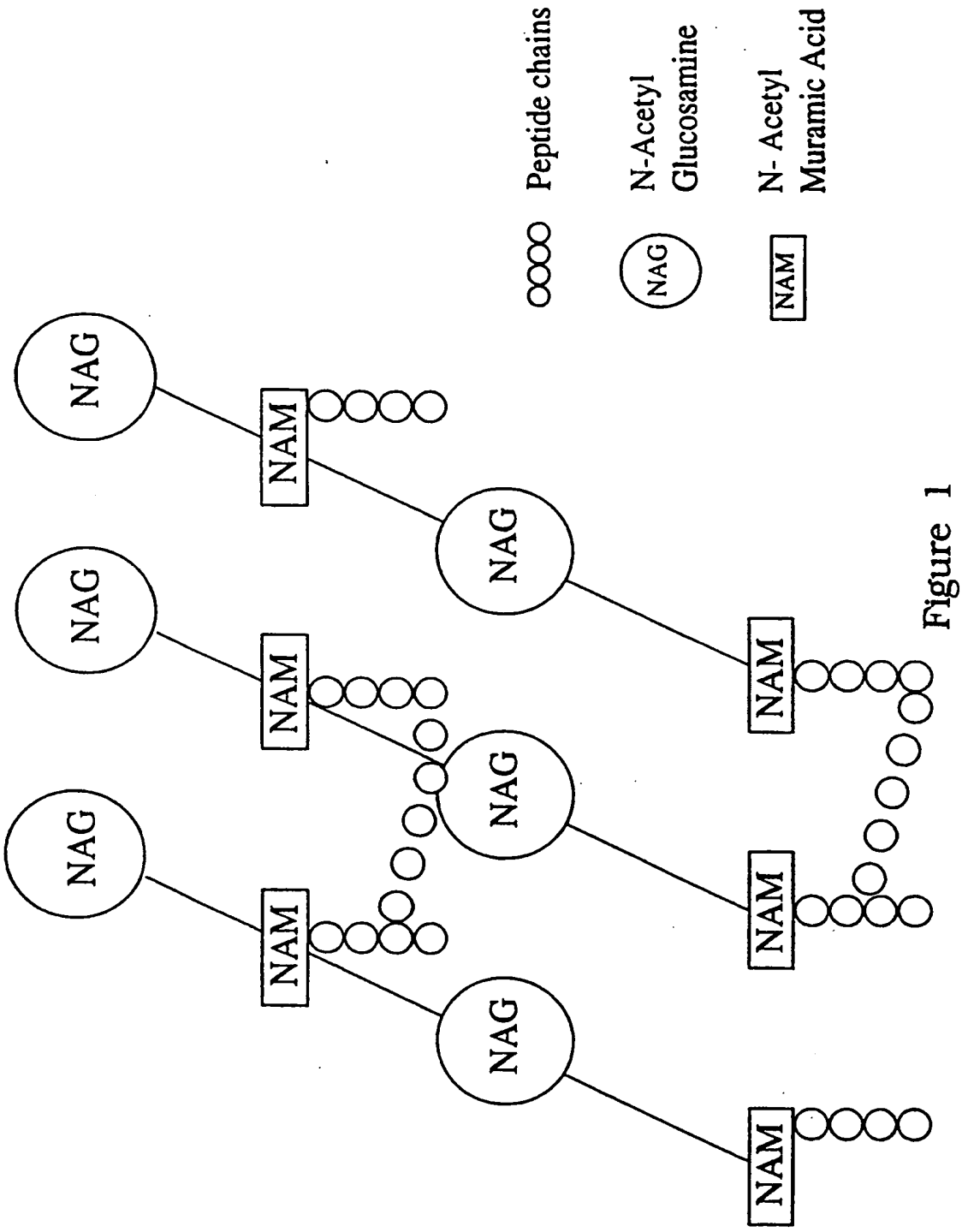


Figure 1

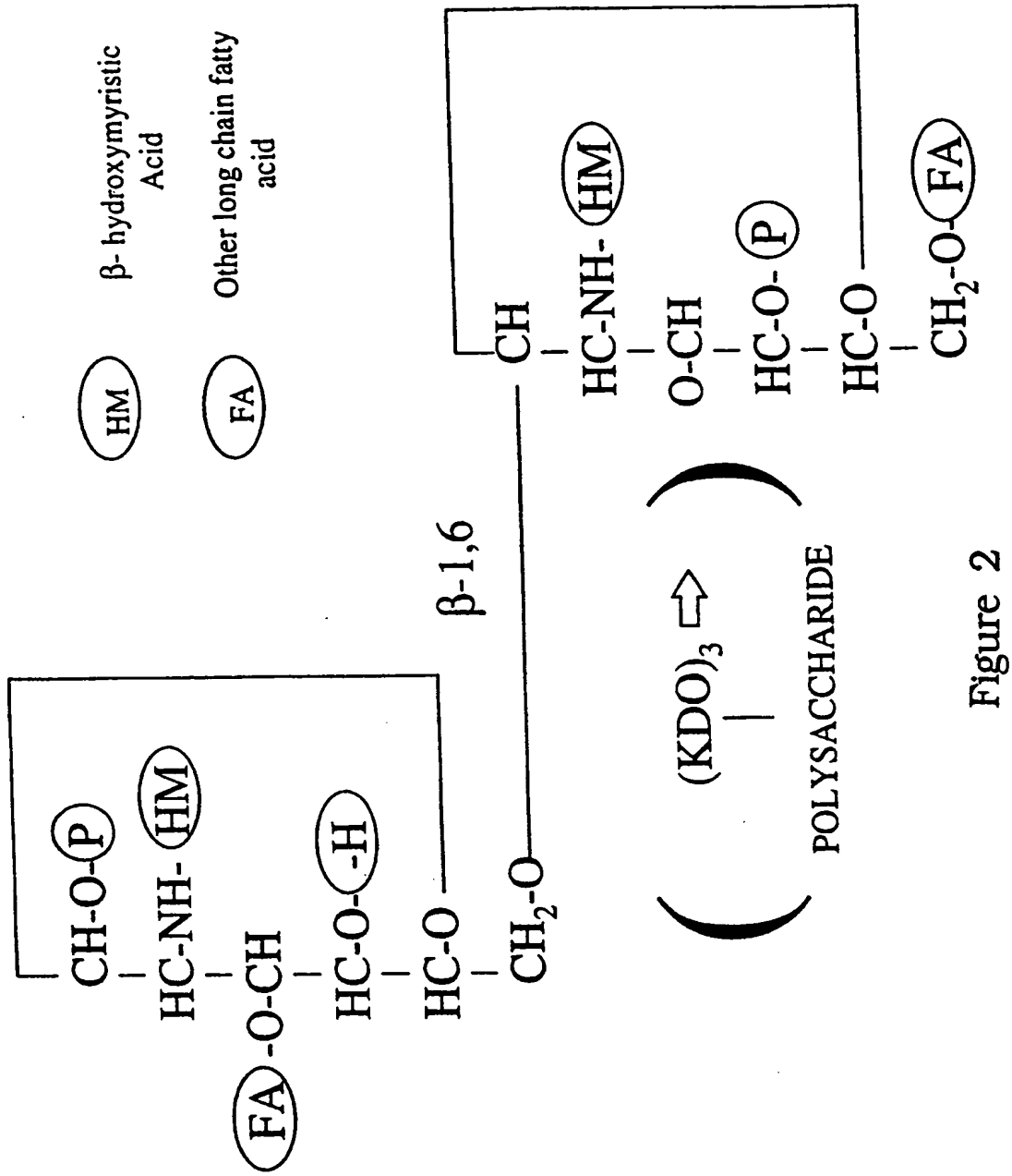
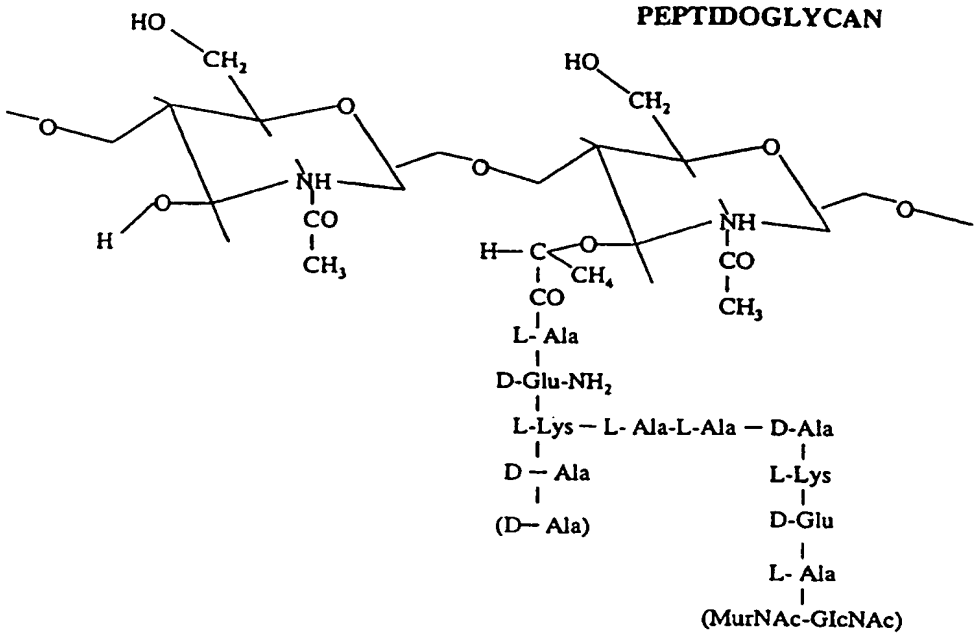

600-1-171 (Sheet 2 of 4)
HM
β- hydroxymyristic Acid
FA
Other long chain fatty acid
CH-O-P
HC-NH- HM
FA -O-CH
HC-O- -H
HC-O
CH2-O
β-1,6
CH
HC-NH- HM
O-CH
HC-O- P
HC-O
CH2-O- FA
(KDO)3
POLYSACCHARIDE

Figure 2

600-1-171 (Sheet 3 of 4)



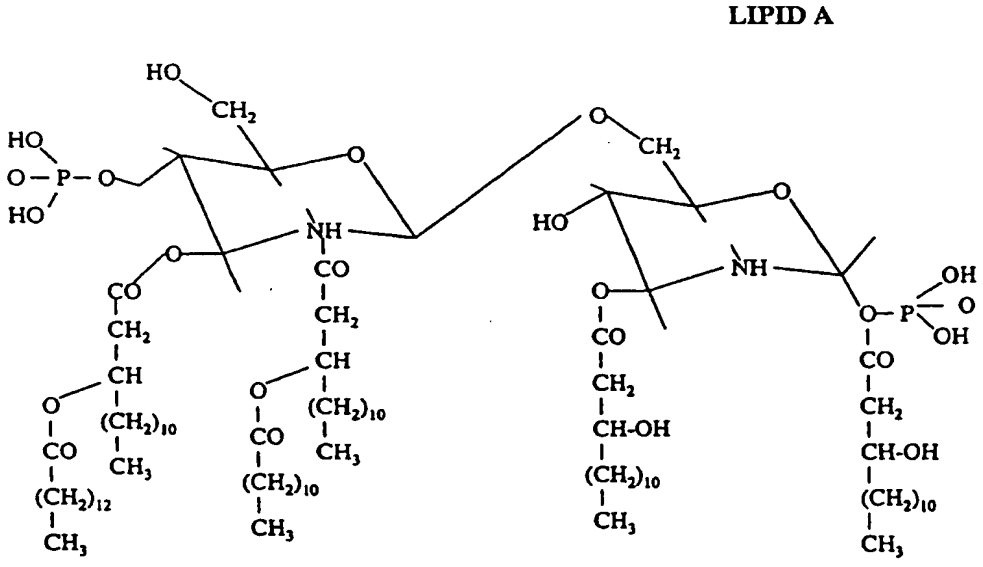


Figure 3

600-1-171 (Sheet 4 of 4)

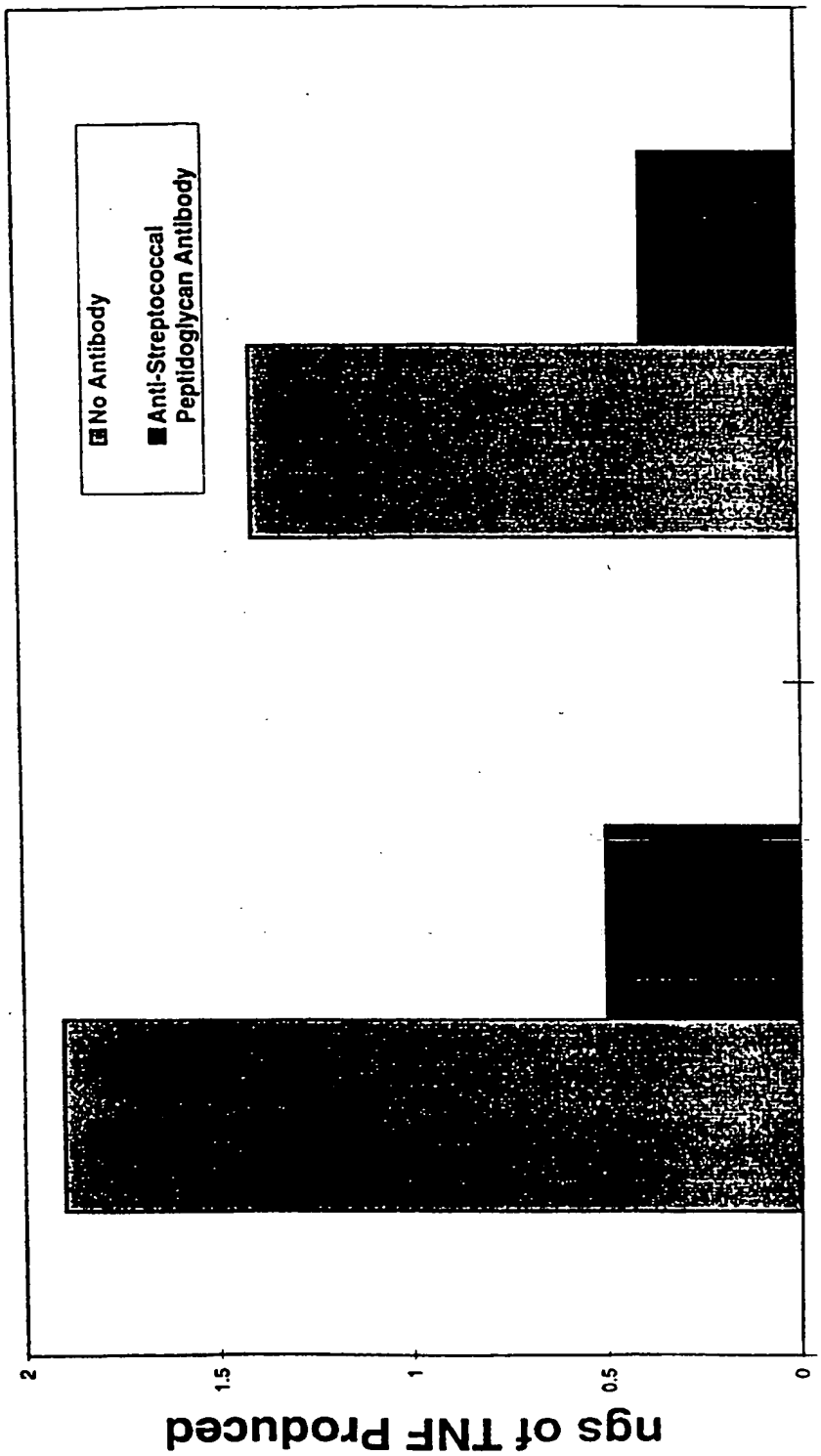


Figure 4

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/US 98/12647

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/00 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 06031 A (IMMUNEX CORP) 2 March 1995 see page 1 - page 6	7,9-12, 15,22,23
A	WO 92 16230 A (UNIVAX BIOLOG INC ;TENG NELSON (US); SADOFF JERALD (US); BHATTACHA) 1 October 1992 see page 2, line 36 - page 18	1-23
A	WO 87 07297 A (UNIV TEXAS) 3 December 1987 see the whole document	1-6,10
A	EP 0 368 466 A (BAXTER INT) 16 May 1990 see the whole document	1-15

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 October 1998	02/11/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Schorsack, B

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INTERNATIONAL SEARCH REPORT

Int. Jonal Application No
PCT/US 98/12647

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 14464 A (YISSUM RES DEV CO ;KUPOT HOLIM HEALTH INSURANCE I (IL); LEVITZKI A) 1 June 1995 see page 1 - page 13, line 25	7-10,14, 15,21-23
A	EP 0 701 818 A (IGEN INC) 20 March 1996 see the whole document	16-18
A	WO 91 07986 A (SCHERING CORP ;FLETCHER STARNES H (US)) 13 June 1991 see the whole document	10
A	REIMER K.B. ET AL: "Immunochemical characterization of polyclonal and monoclonal streptococcus group A Antibodies by chemically defined glycoconjugates and synthetic oligosaccharides" CARBOHYDRATE RESEARCH , vol. 232, 1992, pages 131-142, XP002080496 see the whole document	
A	MACALLAN ET AL.: "Development of a novel TNFalpha Ligand-Receptor Binding Assay for Screening Natchem tm Libraries" J. RECEPT. SIGNAL TRANSDUCTION RES., vol. 17, no. 1-3, 1997, pages 521-529, XP002080497 see the whole document	

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte ional Application No
PCT/US 98/12647

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9506031 A	02-03-1995	AU 5030298 A	05-03-1998
		AU 687436 B	26-02-1998
		AU 7569494 A	21-03-1995
		EP 0715619 A	12-06-1996
		FI 960803 A	22-04-1996
		JP 9503201 T	31-03-1997
		NO 960723 A	23-02-1996
		US 5594106 A	14-01-1997
		US 5629285 A	13-05-1997
WO 9216230 A	01-10-1992	AU 1570792 A	21-10-1992
		CA 2105965 A	16-09-1992
		EP 0575517 A	29-12-1993
		JP 6508606 T	29-09-1994
WO 8707297 A	03-12-1987	US 4929604 A	29-05-1990
		AT 130032 T	15-11-1995
		AU 610509 B	23-05-1991
		AU 7580787 A	22-12-1987
		DE 3751588 D	14-12-1995
		DE 3751588 T	21-03-1996
		EP 0306496 A	15-03-1989
		FI 885464 A,B,	24-11-1988
		JP 4504349 T	06-08-1992
		US 5013661 A	07-05-1991
		US 5200184 A	06-04-1993
EP 0368466 A	16-05-1990	AT 130763 T	15-12-1995
		DE 68924963 D	11-01-1996
		DE 68924963 T	20-06-1996
		ES 2082783 T	01-04-1996
		JP 2134329 A	23-05-1990
WO 9514464 A	01-06-1995	AU 1293595 A	13-06-1995
		CA 2177289 A	01-06-1995
		EP 0731697 A	18-09-1996
		JP 10504014 T	14-04-1998
EP 0701818 A	20-03-1996	AT 135235 T	15-03-1996
		AU 638405 B	01-07-1993

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte .onal Application No
PCT/US 98/12647

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0701818 A		AU 3730489 A	29-11-1989
		AU 643186 B	11-11-1993
		AU 3739389 A	29-11-1989
		DE 68925972 D	18-04-1996
		DE 68925972 T	25-07-1996
		EP 0436545 A	17-07-1991
		EP 0413762 A	27-02-1991
		IL 90200 A	15-04-1997
		JP 3504130 T	12-09-1991
		JP 2772088 B	02-07-1998
		JP 5501948 T	15-04-1993
		WO 8910754 A	16-11-1989
		WO 8910961 A	16-11-1989
WO 9107986 A	13-06-1991	AU 648777 B	05-05-1994
		AU 7179491 A	26-06-1991
		CA 2070424 A	05-06-1991
		DE 69006221 D	03-03-1994
		DE 69006221 T	05-05-1994
		DK 504307 T	21-03-1994
		EP 0504307 A	23-09-1992
		ES 2062762 T	16-12-1994
		FI 922551 A	03-06-1992
		HU 64481 A	28-01-1994
		JP 7047547 B	24-05-1995